PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF THE PLANT EXTRACTS OF *BRASSICA OLERACEA* AGAINST SELECTED MICROBES

Swati Paul* Talha Bin Emran, Didyajyoti Saha, S.M. Zahid Hosen

Department of Pharmacy, BGC Trust University Bangladesh, Chittagong
***Corresponding Author E-mail**: talhabmb@gmail.com, swati946@gmail.com Tel. +8801819942214

Abstract

Brassica oleracea L. is a creeping annual or perennial herb sometimes subshrubs or shrubs. It has been found to have anticancer, antiasthmatic, analgesic properties. It also helps to prevent anemia, infections and many others. In the present study the active phytocomponents of Brassica oleracea were revealed using phytochemical analysis. The antimicrobial activity of Brassica oleracea was studied using well diffusion method. The activity was tested against Aspergillus fumigatus, Citrobacter divergens and Klebsiella pneumonia at different concentrations of 50, 100 and 200µg/disc.

Keywords: *Brassica oleracea, antimicrobial activity, antiasthmatic, anticancer, analgesic*.

Introduction

Herbal medicine involves the use of plants for medicinal purposes. The term "Herb" includes leaves, stems, flowers, fruits, seeds, roots, rhizomes and bark. There can be little doubt that the use of plants for healing purposes is the most ancient form of medicine known. The quest for plants with medicinal properties continues to receive attention as scientists are in need of plants, particularly of ethno botanical significance for a complete range of biological activities, which ranges from antibiotic to anticancerous. Several plants and herb species used traditionally have potential antimicrobial and antiviral properties [1, 2] and this has raised the optimism of scientists about the future of phyto-antimicrobial agents [3].

Several phytochemical surveys have been published, including the random sampling approach which involved some plant accessions collected from all parts of the world. The major chemical substances of interest in these surveys were the alkaloids and steroidal sapogenins, however other diverse groups of naturally occurring phytocomponents such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils etc., have also been reported [4]. There is currently a large and ever expanding global population base that prefers the use of natural products in treating and preventing medical problems because herbal plants have proved to have a rich resource of medicinal properties.

Brassica oleracea L. is an herbs annual, biennial, or perennial, sometimes subshrubs or shrubs, with a pungent, watery juice. It belongs to the Brassicaceae family and common names are- Kohlrabi, Ye Gan Lan, brokolli, folkopi, foreign folkopi etc. About 330 genera and 3500 species: all continents except Antarctica, mainly in temperate areas, with highest diversity in Irano-Turanian, Mediterranean, and W North American regions; 102 genera (eight endemic) and 412 species (115 endemic) in China. The Brassicaceae include many important crop plants that are grown as vegetables (*Brassica, Nasturtium, Raphanus*) and sources of vegetable oils (*Brassica*) and condiments (*Armoracia, Brassica, Eutrema, Sinapis*) . Oils of *Brassica* probably rank first in terms of tonnage of the world's production of edible oils. The family includes many ornamentals in the genera *Erysimum, Iberis* Linnaeus, *Lobularia, Malcolmia*, and *Matthiola*. Of these, only *Lobularia maritima* has become naturalized

in China. The family also includes more than 120 species of weeds. *Arabidopsis thaliana*, which is naturalized in China, has become the model organism in many fields of experimental biology. This plant has a history of use for the treatment of various ailments, e.g., anticancer, antiasthmatic, analgesic etc. Broccoli probably ranks number one on the list of all round anti-cancer vegetables, with broccoli being the subject to the highest amount of scientific and medical research. Broccoli is considered a major deterrent to lung, stomach, mouth, ovarian, breast, cervix, colon and prostate cancer. Since broccoli is high in beta carotene and iron, it may help prevent anemia especially in people who follow a vegetarian diet. Children who have respiratory infections, measles, and gastroenteritis generally experience a significant reduction in their body's levels of vitamin A. The beta carotene provided by broccoli is converted into vitamin A by the body when the body's supplies run low. Thus in theory adding broccoli to a child's diet helps prevent infections.

Materials and Methods

Sample Preparation

The plant selected for present work was *Brassica oleracea* which was collected from Potiya and Reazuddinbazar Bangladesh in March, 2009 at day time. The plant is available in winter season and fresh plants were collected. The collected plant parts were separated from undesirable materials or plant parts. Then the fruits were cutted into very small pieces and kept in the open dry under shadow for 15 days. Then the parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until analysis commenced. About 100 gm of powdered materials was taken in a clean, flat bottomed plastic container and soaked in 700 ml of 99.8% ethanol. The container with its contents was sealed and kept for a period of 14 days. The whole mixture then under went a coarse filtration by a piece of clean, white cotton materials. The filtrate (ethanol extract) obtained was evaporated under ceiling fan and in a tray until dried. It rendered a gummy concentrate of reddish black color. The gummy concentrate was designated as crude extract of ethanol.

Preliminary Phytochemical Screening

The freshly prepared crude ethanolic extract was qualitatively tested for the presence of chemical constituents. These were identified by characteristic color changes using standard procedures Ghani, (2003), Sofowara (1993), Trease and Evans (1989) and Harborne (1973) [5-8].

Antimicrobial Assay

Media Preparation

Bacterial Media (Muller Hinton Media)

36g of Muller Hinton Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petri dishes. The solidified plates were bored with 5mm diameter cork bearer. The plates with wells were used for the antibacterial studies.

Fungal Media (Potato dextrose sugar)

200g of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These

constituents were mixed and autoclaved. The solidified plates were bored with 6mm diameter cork borer. The plates with wells were used for antifungal studies.

Antibacterial activity of the plant extract

The ethanolic extract of 50µg, 100µg and 200µg were tested against two bacterial pathogens namely *Citrobacter divergens* and *Klebsiella pnemoniae*, for their antimicrobial activity. It was demonstrated by well diffusion method.

Antifungal activity of the plant extract

The ethanolic extract of 100, 200 and 500µg were tested against different fungal pathogen *Aspergillus fumigatus* for their antifungal activity. It was demonstrated by well diffusion assay.

Well diffusion method

Antibacterial and Antifungal activities of the plant extract were tested using Well diffusion method [9]. The prepared culture plates were inoculated with different selected strains of bacteria and fungi using streak plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37ºC$\pm$2ºC for 24 hours for bacterial and 25$\pm$2ºC for 48 hours for fungal activity. The plates were observed for the zone clearance around the wells. The ethanol extract was dissolved in sterile distilled water to form dilution such as 50µg, 100µg and 200µg. Each concentration of the plant extract was tested against different bacterial pathogens. It was demonstrated by well diffusion assay [9]. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

Results

The preliminary phytochemical screening of *Brassica oleracea* extract showed the presence of bioactive components like Terpenoids, Flavonoids, Glycosides, Alkaloids, Phenols, Tannins (Table 1).The results of the antimicrobial assay of the ethanolic extract of *Brassica oleracea* indicated that the plant exhibited antimicrobial activity against the tested microorganisms at three different concentrations of 50, 100 and 200µg/disc. The potential sensitivity of the extract was obtained against all the three micro organisms tested and the zone of inhibition was recorded and presented below in the tabulation drawn (Table 2).

Table 1: Phytochemical Screening of ethanolic extract of *Brassica oleracea.*

SL. No.	Tests	*Brassica oleracea* extract
1	Terpenoids	+
2	Flavonoids	+
3	Steroids	-
4	Glycosides	+
5	Alkaloids	+
6	Phenols	+
7	Tannins	+
8	Saponins	-

Table 2: Antimicrobial activity of ethanolic extract of *Brassica oleracea.*

SL. No.	Name of the Organism	Concentration of ethanolic extract added and Zone of inhibition (mm)		
		50µl	**100µl**	**200µl**
1	*Aspergillus fumigatus*	8	10	13
2	*Citrobacter divergens*	4	-	8
3	*Klebsiella pneumonia*	12	15	20

Discussion and Conclusion

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization [10]. Although a significant number of studies have been used to obtain purified plant chemical, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants [11]. In the present investigation, the active phytocomponents of *Brassica oleracea* was studied and further the antimicrobial activity of the plant extract was also tested against three potentially pathogenic microorganisms *Aspergillus fumifatus, Citrobacter diversens* and *Klebsiella pneumonia* at different concentrations of the extract to understand the most effective activity. The maximum zone of inhibition was obtained for *Aspergillus fumigatus* and *Klebsiella pneumonia* at a concentration of 200µg/200µl. While *Klebsiella pneumonia* exhibited good sensitivity against both the concentrations and *Citrobacter divergens* showed medium sensitivity.

From the above studies, it is concluded that the traditional plants may represent new sources of anti-microbials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

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